anfr. nucl. iii., lævibus, tumidis, apice submamillato, subdeclivi; norm. vi., tumidis, suturis impressis; costis radiantibus circ. xiv., haud contiguis, angustis, interstitiis undatis; costulis rotundatis, spiralibus, in spira iv., quarum postica multo minor, supercurrentibus, ad intersectiones subnodosis; costulis circa basim subrotundatam iv., haud decussatis; apertura subquadrata; columella haud truncata, obtuse angulata; labro acuto, a costulis indentato; labio inconspicuo. Long. ·21, long. spir. ·16, lat. ·06 poll., div. 20°.

[To be continued.]

XLV.—*Histological Researches on the Formation, Development, and Structure of the Vegetable Cell.* By Prof. H. Karsten.

[Continued from p. 435.]

§ VII.

The Development of Pollen.—Historical notes.—Origin of pollen, in *Althæa rosea*, from endogenous free cells with prolongations inwards of the thickened walls of the mother cell.—Development of aculei on the surface of pollen-grains.

There is scarcely a vegetable tissue whose development has been more frequently investigated than pollen. Adolphe Brongniart (Annales des Sciences Naturelles, 1827; Génération et Développement de l'Embryon, 1827) was the first to observe that, in the congeries of cells in the anthers of *Cobæa*, the pollen-cells originated in fours. Mirbel made a more special study of the development of the pollen of *Cucurbita* (Recherches sur la Marchantia, 1833). He found that the granular contents of the mother cell of the pollen became divided into four portions by the inward growth of ridges from the sides of the cell towards the centre, and that subsequently the outer surface of each segment became hardened so as to form a smooth skin, within which a second membrane was in process of time produced. The very similar construction of spores and their development occupied the attention of Mohl the same year (Entwickelung und Bau der Sporen der kryptogam. Gew., Flora, 1833). The first appearance of the spores of *Riccia* and *Anthoceros* was recognized by Mohl under the form of four small collections of granules, each of which became enveloped by a delicate membrane. These four masses contained in each cell assumed by mutual pressure a three-sided, obtusely pyramidal form, whilst their fourth side, lying in contact with the parent cell-wall, acquired a convex outline.

Subsequently, Nägeli (Zur Entwickelungsgeschichte des Pollens, 1842) having extended and tested the application of Schleiden's theory of cell-formation to the development of the pollen-cell, and Unger (Ueber merismatische Zellenbildung bei der Entw. des Pollens, 1844) having published his observations upon the formation of the pollen-cells, Mohl felt himself compelled, as a result of his repeated investigations of cell-formation, to assume, like Mirbel and Unger, that, in the development of spores and pollen-cells, the division of the cell by constriction is combined with free-cell-formation (Vegetab. Zelle, 1851, p. 220).

In my investigations on the organic cell I included the development of the pollen-cells of plants of different families; and the results arrived at differed from all others in these material points:—that the pollen-cell, which consists of a complex system of endogenous cells, is developed freely within the pollen mother cell; that the membranes of the numerous cell-nuclei and nuclear corpuscles contained in the pollen mother cells become themselves extended as the coverings of the pollen-cell, a new vesicle, which enlarges to form the nucleus, being formed in them, and in this again the nuclear corpuscle makes its appearance as a microscopic vesicle; so that the origin of the nuclear corpuscles does not precede the formation of the membrane of the nuclear cell, nor is the production of the pollen-cells dependent upon an antecedently formed cell-nucleus. (De Cella vitali, 1843, p. 37, tab. 1 *a–i.*)

However, I was not at that time prepared to encounter the various scruples and objections which these opinions called forth; consequently the brief statement then put forth, and the simple nature of the illustrations given, did not suffice to meet those objections.

Indeed it is a very difficult matter to deduce the law of cell-formation from the history of the development of pollen, inasmuch as these cells, in the course of their development, are more filled than others with opake material, and consequently their growth cannot be made the subject of direct observation, but the course of development must be gathered from a comparison of many specimens. Hence it is that the observer is in this case more exposed to error than with the simple cellular plants.

Still it seems to me that we must not pass over this much-debated and still imperfectly elucidated subject; and I will rather endeavour to prove, from what seems to me the most difficult object of investigation (which indeed most illusively represents the phenomena of constriction and fold-formation), that even the processes here taking place may be explained in accordance with the general law of free-cell-formation. I refer to those pollen-cells of Dicotyledonous plants whose primary cells (special mother cells of Nägeli), as also the membranes of their mother cells, become much thickened and often laminated during the earlier development of the secondary and subsequent generations of the true pollen-cells.

Of these I select the *Althæa rosea* as the best-known example: its June buds, in rapid evolution, before the opening of the first flowers, furnished me with my best material. The later flower-buds, which vegetate more languidly, and perhaps scarcely attain their complete development, are not suited for this investigation. The buds, when taken from the plant, must be examined immediately.

The figures given in Plate V. may be appealed to in illustration, and save description. Figure 13 exhibits the eight large thick-walled cells which occupy the median line of each of the two compartments of the young anther of *Althæa rosea*. Each of these large cells was invested with a double membrane, and its cavity was filled with a granular mucilaginous fluid, in which a nucleus containing one nuclear corpuscle could be detected. (The two uppermost cells were emptied in making the preparation.) Around the central nucleus, at a certain stage of development, were four, or more rarely two, spherical clear spaces, of larger or smaller size (13 x). Now and then, also, in the interior of these clear spaces, a central vesicle, indicative of their cellular nature, was visible.

When these endogenous cells do not yet shimmer through the turbid granular plasma, they may not unfrequently be recognized during the gradual action of water upon their mother cells, especially when the latter are still enclosed (as in fig. 13) in their common mother cell. This action of the water consists not so much in a solution of the plasma as in an extension of the membranes of the enclosed cells, by which means the contrast between their cavities filled with turbid mucilaginous fluid and the surrounding granular plasma is rendered more striking.

After this cambial cell-mass has been acted upon by the water for some time, and the cavities within the mother cells have thereby acquired increasing clearness of outline, they suddenly vanish, usually all four at the same instant, and rarely one later than the rest and after acquiring still more distinctness. To all appearance, the tension of the cell-membranes by endosmosis attains its maximum, and then the cells collapse and disappear, their contents intermingling with those of the mother cell.

That these clear spaces, which are seen to enlarge by imbibition of water under the eye of the observer, do actually possess membranous walls, and constitute actual cells capable of endosmosis, is the less doubtful, as a cell-nucleus is sometimes observed in them. And that they may be four-cell-nuclei incapable of alteration in the normal course of growth, is disproved by the different size of these cells in different mother cells, even in the same anther. By the agency of diosmotic fluids, the secondary pollen-cell detaches itself from the primary cell, and contracts into an irregularly shaped or lobed body upon the granularly cellular contents.

It is at a somewhat later stage of development that these large mother cells of the pollen-cells detach themselves singly from their compartment (fig. 9). The central nucleus is then still recognizable; but its nuclear corpuscle is seen no longer, or with difficulty. The nucleus is situated between four delicate-walled cells, the peripheral portions of the membranes of which are closely applied to the mother cell.

The membrane of the mother cell is more thickened, rather unequally and in laminæ; at that part above the septal wall of the two endogenous cells it is rather thicker, and at a later period its thickness becomes still more decidedly pronounced.

By means of solutions of salts, of sugar, &c., it is possible, even at this stage of development, to separate the contents of the daughter cells from the still delicate primary membrane. The contents of each cell separately form a more or less spherical and smooth mass, whilst the four together constitute a lobed body seen within the centre of the mother-cell, which is divided into compartments by very delicate membranes. These four daughter cells behave, when treated with reagents, in an apparently similar or identical manner with those of *Cladophora* already described. Here also the four daughter cells coherent in the centre may be isolated by the partial solution of their primary cell-walls by reagents; and if the mother cells were injured by cutting, they are, although but rarely, pressed out from it, nearly in the form in which Mohl represented this 'Vegetable Cell' (taf. i. fig. 10).

The granular contents of the secondary pollen mother cells external to the daughter cells either vanish entirely, or a mucilaginous-looking material appears in their stead around these cells, in which case the delicate envelopes of the daughter cells look like the inner contour of a thick-walled cell. In consequence of these changes the pollen mother cell acquires a great similarity to the next phase of development, in which, instead of the central nucleus, a gelatinous mass occupies the centre of the cell betwixt the four daughter cells (fig. 11). This breaking-up of the regenerating nucleus of the cell (nuclear cell) is a sign of the termination of the individual development of the mother cell. The contiguous walls interposed between the four endogenous and much-distended cells form between them apparently simple and very delicate laminæ, which appear to subdivide the cavity of the mother cell then filled with opalescent mucous fluid.

It is this state of things which especially caught the attention of former observers, and which they represented as constituting the first indications of the special pollen-cells.

Unger (Merismatische Zellenbildung bei der Entwickelung des Pollens, 1844) thus refers to the subject:—"At first some thin delicate striæ make their appearance within the mother cell, which can, by making the cell revolve, be proved to be nothing else than transparent walls which divide the granular mass into several portions. They are so fragile that they dissolve in water."

These statements of Unger are entirely correct, supposing that the word "first" applies to the ready formed septa; but even in this condition the delicate membranes appeared to me in the end to yield to the solvent energy of the water.

And in fact, if we will not call in the aid of analogy in the interpretation of the phenomena, it seems impossible to prove that those free cells enveloped by the plasma within the pollen-mother cell and apparently soluble in water, and which may be recognized of different sizes within the unequally developed pollen mother cells of the same anther, form, by the mutual apposition of their enlarged membranes simultaneously with the assimilation of the plasma, the line-like and exceedingly delicate septa (whose double nature can very rarely be detected), by which, in a subsequent stage of development, the turbid cell-juice of the pollen mother cell is subdivided.

The conditions here prevailing do not allow, as in *Œdogonium*, of the actual and continuous observation of the growth of the free endogenous cells, the assimilation of the cell-juice enveloping them, and the formation and increase of their contents. Nevertheless I consider that we are perfectly justified in inferring, from a certainly recognized fact, the occurrence of another similar one, although the latter cannot be observed with the same certainty; and I therefore assume that even the delicate septa, which at a certain stage of development divide the pollen mother cells, are the walls of those free cells which may be detected in them in other similar and earlier states.

This opinion coincides with that of Mohl as expressed in his first-quoted essay on the development of spores.

Moreover in spores, just as in pollen mother cells, four free cells are present, by the expansion of which so as to fully occupy the mother cell the septa in all probability originate.

That these septa are not simple, as supposed by Unger (because even in this very young state they cannot be split by the application of diosmotic fluids), but that they consist of two membranes belonging to two approximated special mother cells of the pollen, was maintained even by Nägeli, although he did not see the gradual growth of these cells, but, on the contrary, supposed, like Mirbel, that the membrane is produced by the solidification of the peripheral layer of the four portions into which the contents of the mother cell are divided.

In anthers of a rather greater age, the thickening of the membranes of the daughter cells forming the septa commences after the secondary membrane of the mother cell has become considerably thickened and acquired seam-like elevations, like those of the cells of collenchyma, between the pollen-cells which are extending themselves peripherally.

This thickening is first seen at the periphery, where they and the mother cells are in contact, and in the next place at the centre, where the cell-nucleus was replaced by the mucoid mass. The boundary-line is at first difficult of detection, owing, without doubt, to the presence of fluid occupying the intercellular spaces. This is probably the reason why Mirbel considered this portion of the thickened primary pollen-cells as the wall of the pollen mother cell itself (fig. 12).

When the thickening has further advanced, so as to occupy the entire extent of the cell-membrane, the boundary-line of the contiguous thickened membranes again becomes visible. The origin and the growth of the secondary and succeeding cells of the pollen-cell cannot be traced with certainty.

The gristly thickened membranes of the pollen mother cell, together with those primary ones of the pollen-cell connected with them (Pl. V. fig. 7), become absorbed in the future course of the formation of the pollen. When exposed for some time to the action of water in the state of greatest thickening, and therefore probably at the commencement of resorption, they are burst asunder by the pollen-cells, which then swell forth at those points where the thickening is least,—that is to say, upon the peripheral vertical lines of the pollen-cells.

The primary cartilaginous thickened cells of the pollen-cells then remain behind, in connexion with the similarly collenchymatose-looking membranes of the mother cell, in the shape of empty envelopes (fig. 10).

The still very thin-walled membrane of the pollen-cells, thrust out in this manner (the secondary cells representing in situation the primary membrane), extrudes from the often comparatively small rent, and, by the continuous imbibition of water, attains eventually double its original diameter. The great elasticity of the young and unthickened cell-membrane is displayed in a remarkable manner during this expansion: it is often squeezed through an aperture scarcely one-fourth its own diameter; but, after having effected its escape, it resumes its dimensions and globular form. In immediate contact with its inner wall there is a layer of minute vesicles, which are rendered more evident by the action of a dilute solution of chloride of calcium. If a rather more concentrated solution of this salt be employed, the entire layer of vesicles, together with a delicate membrane by which it is enveloped, and which lines the somewhat thicker external cell-wall, is separated from the latter, which then appears nearly smooth and structureless, or marked with small paler points, which appear to be the impressions of the vesicles previously closely applied to it (fig. 18).

In a rather younger state, these vesicles are so small that no cavity is discernible within them. By endosmotic action, these vesicles, along with the cell-membrane surrounding them, become detached from the outer wall (figs. 10 & 11), which is then seen to be completely structureless and homogeneous. In somewhat older conditions, on the contrary, the membrane of the secondary cell, with the layer of vesicles adherent on its inner surface, are no longer separable by any such means from the mother cell, nor are the vesicles themselves expansible by endosmotic action. These latter, indeed, appear in intimate union with the two superimposed cell-membranes, and exhibit themselves on the surface of the mother cell in the form of small warts or tubercles; and these again, in cells still more mature, assume the character of prickles, such as are seen distributed over the surface of the pollen-cells of *Althæa* and of other Malvaceæ (fig. 17). Simultaneously with this outgrowth of prickles, the collenchymatically thickened wall of the mother and primitive mother cells are absorbed. These, therefore, resemble in this respect the thickened membranes of the true collenchyma-cells, like which also they have the function of collecting nutriment for the younger endogenous cells.

At the period when the tubercles first make their appearance on the surface, the membrane of the mother cell (originally the secondary cell) is coloured blue by iodine after contact for some time with chloride of calcium, but not at an earlier or later stage of growth.

The existence of free daughter cells within the pollen mother cells, and the origin of septa by the coming into contact of their enlarging primary cell-membranes, are more readily observed in Monocotyledons than in the Malvaceæ, the mucilaginous juices of which render examination difficult.

The pollen of *Fuchsia* is especially interesting, and the history of its development easily followed out. Moreover the nature of the interposed corpuscles, as I have stated in my essay on the Sexual Life of Plants (p. 25 *et seq.*), can be made out in it, owing to frequent aberrations in structure under manifold forms.

[To be continued.]